

FIGURE 1A. Drug Screening Report for TBT, Lot ARB-ID#99-332

VEHICLE	634 µg /ml in EtOH				UNIT mg					
LOT #1	TRIAL #1									
HSV-1 (HFF Cells)										
CPE Inhibition – MCG/ML										
09/17/98	EC50 >10	CC50 >10	SI = 0	ACV EC50 = 1.2						
Plaque Reduction – MCG/ML										
10/19/98	EC50 = 5.9	CC50 >10	SI >1.6	ACV EC50 = 0.3						
HSV-2 (HFF Cells)										
CPE Inhibition – MCG/ML										
09/17/98	EC50 = 2.0	CC50 >10	SI >6.0	ACV EC50 = 1.9						
Plaque Reduction – MCG/ML										
10/19/98	EC50 = 0.9	CC50 >10	SI >11.1	ACV EC50 = 0.1						
HCMV (HFF Cells)										
CPE Inhibition – MCG/ML										
10/01/98	EC50 = 5.0	CC50 >10	SI >2.0	GCV EC50 = 0.06						
Plaque Reduction – MCG/ML										
/ /	EC50	CC50	SI	GCV EC50						
MCMV (HFF Cells)										
Plaque Reduction – MCG/ML										
/ /	EC50	CC50	SI	CCV EC50						
VZV (HFF Cells)										
CPE Inhibition – MCG/ML										
/ /	EC50	CC50	SI	ACV EC50						
Plaque Reduction – MCG/ML										
/ /	EC50	CC50	SI	CCV EC50						

Cytopathic Effect Inhibition and Plaque Reduction Assays For HSV-1, HSV-2, HCMV, MCMV, and VSV, in HFF cell cultures.

FIGURE 1B. Drug Screening Report for TBT, Lot ARB-ID#99-332

EBV						
VCA – MCG/ML (In Daudi Cells)						
10/16/98	EC50 = 1.4	CC50 = 0.21	SI = 0	ACV EC50 = 1.8		
EBV DNA – MCG/ML (In Daudi Cells)						
/ /	EC50	CC50	SI	ACV EC50		
Toxicity Assays						
Neutral Red Uptake – MCG/ML (In Stationary HFF Cells)						
09/17/98	CC50 >10	ACV IC50 >100	GCV IC50 >100			
Cell Proliferation – MCG/ML (In Rapidly Growing Daudi Cells)						
10/16/98	IC50 <0.08	ACV IC50 >50				
Cell Proliferation – MCG/ML (In Rapidly Growing HFF Cells)						
10/16/98	IC50 0.02	ACV IC50 >100	GCV IC50 40.0			
Comments and Recommendations: HCMV PR. This compound is problematic.						

Cytopathic Effect Inhibition and Plaque Reduction Assays EBV in DAUDI cell cultures;
Toxicity Assays in HFF and DAUDI cell cultures.

FIGURE 2. HSV ELISA METHOD

1. Remove the growth media HFF cells.
2. Add 100 µl of MEM to the 6 cell control wells.
3. Add 50 µl of the diluted virus to the remaining wells.
 - A. Herpes simplex virus type 1, ATCC # V-733, strain F. HSV-1 (5/3/96), one plate. Dilute stock 1:2000, 2.0 µl in 4.0 ml MEM.
 - B. Herpes simplex virus type 2, ATCC # VR-734, strain G. HSV-2 (5/3/96), one plate. Dilute stock 1:2000, 2.0 µl + 4.0 ml MEM.

Clinical strains.

1. Specimen # 1. HSV-1, December 9, 1997, eye. Dilute stock 1:500, 8.0 µl plus 4.0 ml of MEM.
2. Specimen # 2, HSV-1, September 30, 1997, oral pharynx. Dilute stock 1:50, 60 µl plus 3.0 ml of MEM.
3. Specimen # 3, HSV-2, Nov. 26, 96, oral pharynx. Dilute stock 1:500, 8.0 µl plus 4.0 ml of MEM.
4. Specimen # 4, HSV-2, Dec. 27, 96, ETT. Dilute stock (May 27, 98) 1:100, 40.0 µl plus 4.0 ml of MEM.
5. Specimen # 5. HSV-2, April 11, 1998, penis. Dilute stock 1:100, 40 µl plus 4.0 ml of MEM.
6. Specimen # 6. HSV-2, April 24, 1998. Dilute stock 1:100, 40 µl plus 4.0 ml of MEM.

4. Add the virus to the plates for one hour and incubate the plates at 37° C.

5. Remove the virus inoculum and add 100 µl of MEM to the virus control wells and add 100 µl of diluted drug to each of the remaining wells.

TBT. Stock TBT contains 624 µg/ml of active component, 10-deacetyl-7-xylosyltaxol in 50% ethanol from Rainbow Research, Inc.

- a. Warm the TBT and 1X MEM to 37° C. The stock TBT was diluted to 4.0 µg/ml by adding 154 µl of TBT to 24,000 µl of 1X MEM.

TBT, µg/ml	4.0	3.0	2.0	1.0	0.75	0.5	0.25	0.1
Vol. Of extract, µl.	6000	6000	4000	2000	1500	1000	500	200
MEM, µl.	-	2000	4000	6000	6500	7000	7500	7800

ELISA RESULTS:

Antibody to HSV-1 was used at a 1:2000 dilution. Antibody to HSV-2 was used at a 1:1500 dilution. Read with dual wavelength, 630 – 490 nm.

FIGURE 3
HSV-1 ELISA CONTROL (ATCC VR-733, STRAIN F)

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.000	0.361	0.069	0.129	0.188	0.373	0.360	0.367	0.309	0.305	
0.000	0.231	0.046	0.093	0.236	0.349	0.357	0.329	0.352	0.362	
0.000	0.304	0.052	0.114	0.206	0.358	0.327	0.291	0.355	0.354	
0.000	0.295	0.033	0.097	0.247	0.338	0.354	0.306	0.316	0.381	
0.000	0.290	0.039	0.098	0.241	0.312	0.301	0.347	0.346	0.361	
0.000	0.303	0.045	0.065	0.203	0.344	0.349	0.333	0.362	0.346	
0.000	0.297	0.047	0.099	0.220	0.346	0.341	0.329	0.340	0.352	
	0.297	0.047	0.099	0.220	0.346	0.341	0.329	0.340	0.352	
		15.9	33.4	74.0	116.3	114.8	110.6	114.3	118.2	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-1					
Control TBT-IC50 = 3 μg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 μg/ml Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 4
HSV-1 ELISA, CLINICAL SPECIMEN #1

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	µg/ml
0.000	0.387	0.147	0.155	0.157	0.331	0.256	0.319	0.272	0.368	
0.000	0.279	0.107	0.166	0.161	0.273	0.235	0.258	0.238	0.370	
0.002	0.256	0.109	0.115	0.203	0.289	0.187	0.258	0.245	0.368	
0.000	0.328	0.110	0.135	0.216	0.295	0.251	0.231	0.274	0.370	
0.005	0.328	0.102	0.142	0.249	0.327	0.318	0.294	0.366	0.314	
0.003	0.344	0.098	0.198	0.204	0.316	0.311	0.324	0.374	0.362	
0.002	0.320	0.112	0.152	0.198	0.305	0.260	0.281	0.295	0.359	
	0.319	0.111	0.150	0.197	0.304	0.258	0.279	0.293	0.357	
		34.7	47.1	61.7	95.2	81.0	87.6	92.0	112.0	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-1					
Specimen #1 TBT-IC50 = 3 µg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 µg/ml Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 5
HSV-1 ELISA, CLINICAL SPECIMEN #2

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.005	0.238	0.016	0.005	0.020	0.048	0.061	0.029	0.029	0.062	
0.002	0.086	0.005	0.019	0.050	0.053	0.054	0.080	0.080	0.056	
0.009	0.111	0.010	0.000	0.000	0.033	0.027	0.104	0.104	0.077	
0.006	0.141	0.034	0.011	0.022	0.065	0.059	0.059	0.059	0.109	
0.012	0.186	0.002	0.057	0.110	0.061	0.087	0.108	0.108	0.086	
0.006	0.196	0.018	0.039	0.017	0.031	0.087	0.090	0.090	0.120	
0.007	0.160	0.014	0.022	0.037	0.049	0.063	0.078	0.078	0.085	
	0.153	0.008	0.015	0.030	0.042	0.056	0.072	0.072	0.078	
		4.9	9.9	19.5	27.3	36.5	46.8	46.8	51.2	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-1					
Specimen #2 TBT-IC50 = 0.25 μg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 μg/ml Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 6
HSV-2 ELISA CONTROL (ATCC VR-734, STRAIN G)

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	µg/ml
0.007	0.172	0.019	0.053	0.033	0.098	0.065	0.088	0.179	0.244	
0.007	0.148	0.016	0.049	0.074	0.079	0.108	0.125	0.150	0.201	
0.005	0.147	0.024	0.014	0.117	0.066	0.086	0.079	0.113	0.245	
0.015	0.169	0.044	0.033	0.060	0.070	0.097	0.107	0.124	0.203	
0.029	0.231	0.026	0.045	0.073	0.068	0.080	0.108	0.155	0.223	
0.008	0.148	0.015	0.031	0.053	0.067	0.103	0.085	0.104	0.177	
0.012	0.169	0.024	0.038	0.068	0.075	0.090	0.099	0.138	0.216	
	0.157	0.012	0.026	0.057	0.063	0.078	0.087	0.126	0.204	
		7.7	16.3	35.9	39.9	49.6	55.2	79.9	129.4	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-2					
Control TBT-IC50 = 0.75 µg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 µg/ml Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 7
HSV-2 ELISA, CLINICAL SPECIMEN #3

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.015	0.337	0.119	0.109	0.145	0.290	0.137	0.200	0.264	0.204	
0.008	0.266	0.109	0.105	0.168	0.167	0.129	0.124	0.249	0.248	
0.010	0.207	0.056	0.077	0.154	0.215	0.106	0.158	0.191	0.253	
0.015	0.213	0.079	0.134	0.141	0.171	0.155	0.288	0.183	0.165	
0.027	0.211	0.131	0.090	0.105	0.171	0.122	0.203	.0189	0.168	
0.007	0.217	0.070	0.080	0.059	0.139	0.132	0.110	0.125	0.145	
0.014	0.242	0.094	0.099	0.129	0.192	0.130	0.181	0.200	0.197	
	0.228	0.080	0.086	0.115	0.179	0.117	0.167	0.187	0.184	
		35.2	37.5	50.4	78.2	51.1	73.1	81.7	80.4	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-2					
Specimen #3 TBT-IC50 = 2.0 μg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 $\mu\text{g}/\text{ml}$ Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 8
HSV-2 ELISA, CLINICAL SPECIMEN # 4

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.012	0.190	0.026	0.021	0.059	0.089	0.018	0.066	0.115	0.148	
0.004	0.152	0.018	0.028	0.037	0.059	0.072	0.083	0.106	0.165	
0.030	0.199	0.001	0.042	0.033	0.026	0.052	0.065	0.073	0.195	
0.012	0.174	0.016	0.042	0.046	0.095	0.028	0.064	0.123	0.161	
0.020	0.224	0.033	0.048	0.027	0.031	0.025	0.100	0.078	0.229	
0.010	0.213	0.021	0.025	0.029	0.056	0.046	0.086	0.088	0.152	
0.015	0.192	0.019	0.034	0.039	0.059	0.040	0.077	0.097	0.175	
	0.177	0.005	0.020	0.024	0.045	0.026	0.063	0.083	0.160	
		2.5	11.1	13.4	25.2	14.4	35.3	46.5	90.4	
<u>DATE:</u> 6/24/98					<u>INOC:</u> 6/22/98					
<u>DRUG:</u> TBT					<u>VIRUS:</u> HSV-2					
Specimen #4 TBT-IC50 = 0.25 μg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 μg/ml Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 9
HSV-2 ELISA, CLINICAL SPECIMEN #5

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	μg/ml
0.016	0.220	0.041	0.026	0.052	0.121	0.134	0.109	0.159	0.104	
0.013	0.219	0.017	0.045	0.048	0.084	0.078	0.084	0.130	0.114	
0.003	0.128	0.031	0.067	0.029	0.069	0.071	0.043	0.107	0.120	
0.033	0.210	0.010	0.051	0.077	0.061	0.062	0.055	0.184	0.100	
0.022	0.256	0.025	0.068	0.094	0.062	0.083	0.104	0.131	0.200	
0.036	0.167	0.018	0.083	0.137	0.131	0.111	0.107	0.134	0.141	
0.021	0.200	0.024	0.057	0.073	0.088	0.090	0.084	0.141	0.130	
	0.180	0.003	0.036	0.052	0.068	0.069	0.063	0.120	0.109	
		1.8	20.1	29.2	37.6	38.6	35.2	67.0	60.9	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-2					
Specimen #5 TBT-IC50 = 0.5 μg/ml										

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 $\mu\text{g}/\text{ml}$ Taxus Brevifolia Tincture (TBT) has been added.

FIGURE 10
HSV-2 ELISA, CLINICAL SPECIMEN # 6

CC	VC	4	3	2	1	0.75	0.5	0.25	0.1	µg/ml
0.013	0.125	0.024	0.022	0.015	0.014	0.045	0.018	0.013	0.039	
0.012	0.154	0.028	0.017	0.111	0.011	0.077	0.037	0.055	0.050	
0.019	0.146	0.015	0.061	0.061	0.019	0.009	0.009	0.079	0.103	
0.017	0.128	0.043	0.021	0.024	0.124	0.012	0.037	0.058	0.075	
0.017	0.128	0.045	0.024	0.023	0.102	0.032	0.058	0.061	0.049	
0.033	0.092	0.029	0.017	0.033	0.035	0.061	0.021	0.082	0.077	
0.019	0.129	0.031	0.027	0.045	0.051	0.039	0.030	0.058	0.066	
	0.110	0.012	0.009	0.026	0.032	0.021	0.012	0.040	0.047	
		11.0	7.7	23.6	29.3	18.9	10.4	35.8	42.6	
DATE: 6/24/98					INOC: 6/22/98					
DRUG: TBT					VIRUS: HSV-2					
					Specimen #6 TBT-IC50 = 0.1 µg/ml					

Column headings for the ELISA are culture control (CC) representing uninfected cell culture; Viral Control (VC), representing infected cell culture; 4-3-2-1-0.75-0.5-0.25-0.1 are viral infected cell cultures to which 4 to 0.1 µg/ml Taxus Brevifolia Tincture (TBT) has been added.